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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

A Scientific Meeting was held at The Queen's University of Belfast on Wednesday–Friday, 8–10 September 1993, when the following papers were presented.

Type A behaviour and diet. By M. E. BARKER[1], K. A. THOMPSON[2] and S. I. MCCLEAN[2], [1]Centre for Human Nutrition, University of Sheffield, Sheffield S5 7AU and [2]Centre for Health and Social Research, University of Ulster, Coleraine BT52 1SA

Type A behaviour, characterized by time-driven conduct, a strong orientation towards work responsibilities or task completion and easily provoked hostility, has been positively associated with incident and prevalent ischaemic heart disease (IHD) (Barker et al. 1989). However, little is understood of the relationship between Type A and diet, which may mediate in the association between Type A and IHD. Gallacher et al. (1988) reported that Type A score was related to meal size and meal frequency in a cohort of 532 men; however, there were no trends with absolute nutrient intake.

The present investigation explores the relationship between Type A behaviour, assessed using the Bortner questionnaire, and nutrient intake, assessed using 7 d weighed records, for 551 men and women (aged 16 to 64 years) from Northern Ireland. The relationship between Type A behaviour and food pattern (Barker et al. 1990) is also examined. Statistical analysis was carried out using partial correlation coefficients controlled for age, household socio-economic group and smoking habit.

In men there were positive correlations between Type A score and absolute intakes of energy, protein, fat, carbohydrate and sugar; these were significant for protein intake ($r +0.16$, $p<0.01$), and fat intake ($r +0.13$, $p<0.05$). In women, Type A score was positively related to energy and all proximate constituents; the correlation was only significant for sugar ($r+0.11$, $p<0.05$). NSP intake was positively correlated to Type A score in men and women, however the correlations were not significant.

When Type A score was examined in relation to food pattern it was found that in both men and women the 'traditional' and the 'meat and two veg' behaviours were negatively associated with Type A. In contrast, the 'cosmopolitan' and the 'convenience' behaviours were positively associated with Type A. The correlation between Type A and the 'convenience' pattern was significant ($r +0.13$, $p<0.05$) for both men and women. This pattern of eating has beer, chips, sauces, soft drinks, nuts and cheese as priority foods. This association of Type A behaviour with a convenience regimen concurs with the finding of Gallacher et al. (1988) of an association between small and frequent meals and Type A personality.

Although the magnitude of these associations is small, the results demonstrate that personality and diet are related. However, their relevance to the development of IHD remains speculative.

Barker, M.E., McClean, S.I., McKenna, P.G., Reid, N.G., Strain,J.J., Thompson, K.A., Williamson, A.P. & Wright M.E. (1989). Diet, Lifestyle and Health in Northern Ireland. Coleraine: University of Ulster.

Barker, M.E., McClean, S.I., Thompson, K.A. & Reid N.G. (1990). British Journal of Nutrition **64**, 319-329.

Gallacher, J.E.J., Fehily, A.M., Yarnell, J.W.G. & Butland, B.K. (1988). Appetite **11**, 129-136.

Dietary changes in a nationally-representative sample of Irish adults. By M. KEARNEY[1], A. KELLY[2] and M. J. GIBNEY[1], [1]Unit of Nutrition and Dietetics, Department of Clinical Medicine, Trinity Medical School, St. James's Hospital, Dublin 8 and [2]Department of Community Health, University of Dublin, Trinity College, Dublin 2, Republic of Ireland

A quota-controlled, nationally-representative sample of 1401 adults from the Republic of Ireland completed an in-home, interview-assisted questionnaire. The questionnaire included sections on attitude to health, attitude to nutrition, sources of nutritional information, attitude to eating, nutritional knowledge and dietary changes. Information was gathered on self-reported body weight and height, and exercise, smoking and alcohol habits, to calculate a health index for each respondent.

Thepresent study examines the dietary changes reported by the sample. Of the sample, 32% claimed that their diets had changed for the better over the last three years while 61% claimed no change. An ordinal logistic regression model was fitted to the data, which included the variables, overall dietary change, sex, age, social class and educational achievement. It was found that age and educational achievement were significant in predicting the reporting of overall dietary change. Sex was not a significant independent variable; however there was a sex-education interaction effect. Social class was not significant.

To investigate the dietary changes in more detail, the respondents were shown a list of thirteen food types and they were asked if they were now eating more, less or the same amount of each food, compared with 3 years ago, and whether they intended to alter consumption in the future. The figures pertaining to changes in red meat consumption are outlined in the table, as an example:

Percentage reporting "have eaten less" Beef and other Red Meat

	Total	Male	Female
n...	1401	691	710
Total	27	21	32
Age group:			
15 - 35 years	23	17	30
35 - 55 years	28	23	33
55 years +	30	24	35
Education level:			
Primary	22	17	29
Secondary	26	20	32
Tertiary	36	32	41[a]

[a] significant sex-education interaction effect (χ^2 analysis): $p < 0.025$

The table illustrates the age effect on the reporting of dietary change. It is clear that there is a difference between the sexes. As the level of educational achievement increases, so does the total proportion reporting dietary change. Educational achievement also has a within-sex effect, as was expected from the regression analysis.

The present study has outlined the importance of some sociodemographics in determining dietary change.

This project was funded by Nutriscan Ltd. and The Irish American Partnership.

An approach to the formulation of healthy eating advice. By M.A.T. FLYNN[1,2], M.B. CODD[3], D.D. SUGRUE[2] and M.J. GIBNEY[1], [1]Department of Clinical Medicine, Trinity College Medical School, St James's Hospital, Dublin 8, Republic of Ireland. Departments of [2]Cardiology and [3]Epidemiology, Mater Misericordiae Hospital, Dublin 7, Republic of Ireland

Advice that is based on national dietary data often does not relate to the different dietary patterns that exist within a population. The present study examines the dietary intakes of a group of healthy, working women to research the possibility that population subgroup data may provide a more useful basis for the formulation of advice specifically aimed at effecting a reduction in the saturated fat intakes of those groups.

A total of eighty-three women, aged between 20 and 50 years were recruited from their workplaces, forty-three from socio-economic (SE) classes 1 and 2 and forty from SE classes 5 and 6. Dietary information was collected by a dietitian using the diet history method. The total group was divided into quartiles on the basis of percentage energy intake from saturated fat. This allowed for comparison of the nutrient patterns associated with low and high saturated fat intakes.

Although saturated fat intakes expressed as % of dietary energy were not confounded by alcohol intakes, they were confounded by sugar intakes, particularly non-milk extrinsic sugar intakes which were higher in the women with lower saturated fat intakes. The mean daily intakes of total and non-milk extrinsic sugars in female subjects of low and high saturated fat intakes are shown in the table below:

Saturated fat intake (% dietary energy)....	Lowest quartile(≤ 15.3%)		Highest quartile (> 19.4%)	
n	21		20	
	Mean	SD	Mean	SD
Sugar intake (g/d):				
Total sugars	101.2	18.9	72.5	24.8
Non-milk extrinsic sugars	60.6	31.9	39.0	16.9
Percentage total energy:				
Total sugars	18.7	4.0	13.3	4.2
Non-milk extrinsic sugars	10.9	4.8	7.7	3.2

Several studies have shown an inverse relationship between total fat and sugar intakes, expressed as percentages of dietary energy intakes (DHSS, 1989). The present study now extends this finding for total fat to saturated fat. Nonetheless, the high non-milk extrinsic sugar intakes of the low saturated fat eaters found in the present study were, at 10% of dietary energy, well within the recommended safe range of intake of less than 15-20% (DHSS, 1989) and at the level found acceptable by a more recent review committee (DHSS, 1991).

In conclusion, the present study found no reason to reduce high sugar intakes, but suggests that the role of sugar in rendering a low saturated fat diet acceptable should be considered.

DHSS. (1989). Dietary sugars and human disease. London : H.M. Stationery Office.

DHSS. (1991). Dietary reference values for food energy and nutrients for the United Kingdom. London H.M. Stationery Office.

Perception of Food Allergy in Children aged under 4 Years. By I. KILGALLEN and M.J. GIBNEY, Unit of Nutrition and Dietetics, Department of Clinical Medicine, Trinity Medical School, St. James's Hospital, Dublin 8, Republic of Ireland

In a previous study of the perceived prevalence of food allergy in a group of Dublin families (Sugrue & Kennedy, 1992), children under 4 years were poorly represented in comparison with the proportion of children of this age-group in the 1986 census report (Census 1986 Summary Population Report - 2nd series). Only 8% of the children were aged under four in comparison to 21% in this age-group in the census. Food allergy is known to be more common in young infants and children and so the 8% prevalence rate quoted from the 1991 study may be an under-representation of the true prevalence of perceived food allergies in the population aged under 4 years. The objective, therefore, was to evaluate the perceived prevalence of food allergy in children aged under 4 years.

An interview-assisted questionnaire was designed for use with parents of young children. It covered presence or absence of perceived food allergy, symptoms, foods implicated, infant feeding history and parental background. The parents of 600 children were interviewed in ante-natal clinics and health centres in Dublin city. After entering the coded questionnaires onto a database and performing summary statistics, contingency table analysis by chi-squared tests were used to test for differences between groups.

Of the sample, 12.5% were perceived by their parents to have a food allergy. Neither parental age, education level nor socio-economic group affected perception of food allergy. Hyperactivity was the most common symptom noted, with sweets and sugary drinks being the most commonly implicated foods. Most of the perceived food allergies were diagnosed by the parents by simple avoidance of the suspected foods. Of the parents, 46% perceived food allergy in young children to be either very common or common, with only 7% considering it to be rare. Those perceiving food allergy to be very common were most likely also to perceive their child as food allergic. No difference was found between those children perceived as food allergic and those children not so perceived in terms of breast-feeding history, age of weaning and foods used at weaning.

Although the figure for perceived prevalence of food allergy in young children from the present study is lower than those from other countries, it is higher than the actual prevalence of food allergy - thought to be between 1% and 3% in young children. This may be due to confusion over the true meaning of food allergy and also media attention to the subject. No major nutritional implications were noted. Any energy deficits from cutting sweets or sugary drinks from the diet can be replaced easily. Most of the children were 'allergic' to only one food, the avoidance of which should not lead to any deficiencies. However, most of the parents received no nutritional advice and parents should not be encouraged to restrict their childrens' diets without specialist advice.

Sugrue, S. & N, Kennedy. (1992). *Proceedings of the Nutrition Society* **51**, 68A
Census 1986 Summary Population Report - 2nd series, Central Statistics Office, Ireland.

This project was funded by the National Dairy Council.

The effect of a beta-agonist (clenbuterol) on heat production of beef heifers. By E. F. UNSWORTH[1], C. T. ELLIOT[2], W. J. McCAUGHEY[2] and R. S. PARK[1], [1]Food and Agricultural Chemistry Division and [2]Veterinary Sciences Division, The Department of Agriculture for Northern Ireland Belfast BT9 5PX

The overall response in cattle to dietary administration of clenbuterol has been to reduce body fat content resulting in a leaner carcass. Physiological responses have included elevated heart rate and stroke volume resulting in increased blood flow and O_2 uptake in tissues (Eisemann, Huntingdon, & Ferrell, 1988; Huntingdon, Eisemann, & Whitt, 1990). In the present study the initial and longer-term effect of dietary clenbuterol administration on whole body heat production was examined in fat heifers.

Six mixed breed heifers, mean liveweight 550 kg, were offered *ad lib.* grass silage and 4 kg concentrate.d^{-1} for 2 weeks before and throughout the 24d study. On days 1-4 the animals received no clenbuterol (WOC); on day 5 and for the following 15d the animals received 0.88 mg clenbuterol.d^{-1} in the morning concentrate feed (WC); the clenbuterol was removed from the feed on day 21. Heat production rates, uncorrected for urinary energy excretion, of the animals were measured by indirect respiration calorimetry over two 8d periods, days 1-8 and 17-24 respectively. This enabled the initial response to the drug and its longer-term effect on heat production to be assessed. The mean results obtained are shown in the Table.

Treatments	WOC	WC	WC	WC	WOC	
Days	1-4	5	6-8	17-20	21-24	SED
n	6	6	5	4	4	
CH_4 output ($l.d^{-1}$)	270^{b}	163^{a}	175^{a}	261^{b}	259^{b}	12.6
CO_2 output ($l.d^{-1}$)	3680^{b}	4261^{a}	3990^{ab}	4070^{ab}	3827^{b}	143.1
O_2 consumption($l.d^{-1}$)	3607^{d}	4594^{a}	4328^{ab}	4169^{bc}	3908^{cd}	132.5
Heat production ($MJ.d^{-1}$)	76.2^{d}	95.3^{a}	89.7^{ab}	87.3^{bc}	81.9^{cd}	2.79
RQ	1.02^{c}	0.93^{a}	0.92^{a}	0.98^{b}	0.99^{bc}	0.016

Means within the same row with unlike superscript were significantly different $P<0.05$, ANOVA.

Administration of clenbuterol had an immediate, significant ($P<0.05$) effect on respiratory exchange and, consequently, on heat production which appeared to decline with time (days 5-20) Removal of the drug resulted in a further non-significant decline in heat production (days 21-24) to an intermediate level between that whilst on treatment and the initial (days 1-4) values. The significant ($P<0.05$) decline in CH_4 production on initial exposure to the drug was associated with a marked but transient decline in feed intake in the majority of the animals and in one instance an animal was removed from the experiment on day 7. There were no further significant differences in CH_4 production.

These results confirm, in the whole animal, the increased O_2 uptake observed by Eisemann, Huntingdon, & Ferrell (1988) and Huntingdon, Eisemann, & Whitt (1990) following clenbuterol administration. However, the present study shows that the animal is capable of adapting its metabolism, hence its heat production, to continued clenbuterol treatment. The decline in heat production on withdrawal of dietary clenbuterol suggests that the effect of the drug on overall metabolism was relatively short-lived. The RQ shows that fat catabolism was higher during the treatment period.

Eisemann, J.H., Huntingdon, G.B. & Ferrell, C.L. (1988). Journal of Animal Science **66**, 342-353
Huntingdon, G.B., Eisemann, J.H. & Whitt, J.M. (1990). Journal of Animal Science **68**, 1666-1673.

Chronological change in serum insulin-like growth factor 1 concentrations and the relationship to growth in unweaned male, castrate male and female lambs sired by Texel or Suffolk ram. By A.R.G. WYLIE[1] and D.M.B. CHESTNUTT[2], [1]The Department of Agriculture for Northern Ireland and The Queen's University of Belfast, Newforge Lane, Belfast, BT9 5PX and [2]The Agricultural Research Institute of Northern Ireland, Hillsborough, Co Down, BT26 6DP

Insulin-like growth factor 1 (IGF-1), produced by the liver in response to growth hormone, is a nett promoter of protein anabolism (Lobely, 1993) and is implicated in lean tissue deposition. The current consumer preference for lean meat is of particular importance to lamb production in which carcases are often too fat. In Northern Ireland, lowland lamb production has been largely based on the Greyface (Scottish Blackface x Border Leicester) ewe and Suffolk sire but the use of Texel sires to improve carcase leanness (Croston *et al.* 1987) is increasing.

As part of a wider study of the effects of breed and sex on growth and carcase quality, serum IGF-1 was monitored at 3 week intervals between 8 and 20 weeks of age in unweaned lambs of Greyface ewes mated to Texel (T) or Suffolk (S) rams. Lamb numbers were matched for breed (n 42) and, within breed, for birth status (thirty-six twins; six singles) and sex (twelve each of male (M), castrate male (C), and female (F) twin lambs; three each of M and C single lambs). Twin combinations were also balanced between breeds (six each of M/F, M/C and F/C). Ewes and lambs were put out to grass 1 week after lambing and lambs were weighed and dosed with anthelminthic at each blood sampling. M and F lambs remained sexually immature at the time of last sampling. Serum IGF-1 was determined by a double-antibody radioimmunoassay after cold acid-ethanol extraction of binding protein, analysing all samples from individual lambs in the same assay. IGF-1 overall mean values (ng/ml) are given in the table below

	Texel			Suffolk				
	M	C	F	M	C	F	*n*	SEM
twin	400	347	318	393	311	287	12	16.5
single	516	426	-	368	480	-	3	32.9

Mean serum IGF-1 concentration (ng/ml) was higher at each sampling, and higher overall, in T- than S- sired lambs (371.5 *v.* 343.5; $p<0.05$). Mean IGF-1 was higher in single than twin M and C lambs (447 *v.* 362; $p<0.001$). Twin M lambs had higher ($p<0.001$) mean IGF-1 (396) than either twin C (329) or twin F lambs (302), which were not significantly different, and this difference was not affected by breed or sex of sucking partner. Mean IGF-1 levels fell between first and last sampling dates (385 *v.* 331). The rate of change was greater in single than in twin lambs (-3.16 *v.* -0.75 ng IGF-1/ml per d, $p<0.001$) but was unaffected by breed or sex.

Mean serum IGF-1 (ng/ml) was positively correlated (r 0.576; $p<0.001$) with the rate of liveweight (LW) change but not with mean LW over weeks 8 to 20. The rate of fall of IGF-1 was also positively correlated (r 0.541; $p<0.001$) with the rate of LW change.

IGF-1 is nutritionally regulated such that serum levels are reduced when nutrient intake declines in quantity and/or quality. Accordingly, changes in milk availability and composition as lactation progressed may have dictated the higher IGF-1 levels in single lambs and the fall in IGF-1 overall. The sex ranking (M > C or F) of IGF-1 levels in the present study extends the data of Roberts *et al.* (1990) to unweaned, prepubertal lambs. The higher IGF-1 levels in males and castrates and in all Texel-sired lambs, which produce typically leaner carcases, supports the hypothesis that IGF-1 is involved with lean deposition.

IGF-1 antiserum used in the present study (lot UBK 487) was a gift of the National Institute of Diabetes and Digestive and Kidney Diseases, Maryland, USA.

Croston,D., Kempster,A.J., Guy,D.R. & Jones,D.W. (1987). Animal Production **44**, 99-106.
Lobley,G.E. (1993). Journal of Nutrition, **123**, 337-343.
Roberts,S.A., McCutcheon,S.N., Blair,H.T., Gluckman,P.D. & Breier,B.H. (1990). Domestic Animal Endocrinology 7, 457-464.

Uptake of α-tocopherol in porcine plasma, muscle and adipose tissue. By H. SISK[1], M. MOLLOY[2], P.A. MORRISSEY[1] and D.J. BUCKLEY[2], Departments of Nutrition[1] and Food Technology[2], University College Cork, Republic of Ireland

α-Tocopherol is distributed throughout the body with the highest concentrations in liver, adipose tissue and skeletal muscle (Jensen et al. 1988). However, little information is available on the responses of porcine tissues to long-term administration of α-tocopherol. The purpose of the present study was to investigate the uptake of α-tocopherol in porcine plasma, muscle, subcutaneous fat and kidney fat following dietary α-tocopheryl acetate supplementation. Landrace X Large White pigs were weaned at 24 d, divided into mixed sex groups and assigned at random to one of two diets containing 20 (basal) or 200 mg α-tocopheryl acetate/kg. Pigs were slaughtered at the beginning of the supplementation period (n 8) and at six selected intervals up to 18 weeks (n 4). Tissue samples were taken, stored at -20° and analysed for α-tocopherol by the method of Buttriss & Diplock (1984).

Duration of dietary		Tissue α-tocopherol concentration (μg/g tissue)						
treatment (weeks) ...		0	1	2	3	5	13	18
Plasma	Mean	0.79[a]	1.96[b]	2.20[b]	2.41[c]	3.16[d]	4.50[e]	5.12[e]
	SEM	0.12	0.22	0.23	0.24	0.36	0.40	0.39
Muscle	Mean	1.31[a]	1.51[a]	1.89[b]	2.04[b]	2.42[c]	3.82[d]	4.81[d]
(Longissimus	SEM	0.09	0.09	0.05	0.05	0.14	0.08	0.30
dorsi)								
Kidney Fat	Mean	1.52[a]	3.47[b]	4.64[c]	7.85[d]	13.46[e]	20.31[f]	25.67[g]
	SEM	0.45	0.77	1.25	2.64	2.78	3.11	3.97
Subcutaneous Fat,	Mean	2.56[a]	2.65[a]	3.98[b]	4.96[c]	6.55[d]	13.94[e]	18.08[f]
(Upper layer)	SEM	0.52	0.71	1.14	1.32	1.35	1.23	2.54
Subcutaneous Fat	Mean	3.61[a]	3.28[a]	6.30[b]	8.90[c]	13.40[d]	21.43[e]	25.68[f]
(Lower layer)	SEM	0.91	0.92	1.05	1.23	1.31	1.46	2.07

[a-g] Mean values in the same row with unlike superscripts are significantly different (*t* -test): $P < 0.05$.

A marginal increase was observed in the α-tocopherol concentration of tissues from pigs fed the basal diet (data not shown). For pigs fed on the diet supplemented with 200 mg α-tocopheryl acetate/kg, α-tocopherol levels increased with increasing supplementation-time up to 13 weeks in all tissues examined (Table). In the case of plasma and muscle an upward trend was observed between the results for week 13 and week 18, but the values were not significantly different. Kidney fat and subcutaneous fat demonstrated a further significant increase between week 13 and week 18. It is apparent that mean α-tocopherol levels varied between the tissues analysed. α-Tocopherol was found in highest concentrations in kidney fat followed by subcutaneous fat, lower layer >subcutaneous fat, upper layer > muscle.

Buttriss, J.L. & Diplock, A.T. (1984). Methods in Enzymology **105,** 131-138.

Jensen, M., Hakkarainen, J., Lindholm, A., & Jonsson, L. (1988). Journal of Animal Science **66,** 3101-3111.

Effect of dietary oil quality and α-tocopherol supplementation on the oxidative stability of broiler tissues. By K. GALVIN[1], P.A. MORRISSEY[1] and D.J. BUCKLEY[2], Departments of [1]Nutrition and [2]Food Technology, University College, Cork, Republic of Ireland

Oxidation of poultry feed constituents can result in a number of adverse effects including depressed growth and, in extreme cases, encephalomalacia. It is thought that oxidation products are absorbed and incorporated into tissues, resulting in decreased oxidative stability (Izaki et al. 1984). Dietary supplementation with α-tocopherol has been shown to be an effective means of improving the oxidative stability of tissues. The purpose of the present study was to investigate the effect on oxidation in chicken tissues of feeding oxidized dietary oil together with different levels of dietary α-tocopherol.

Cobb 500 broilers (1-d-old; *n* 144) were randomly divided into six groups and fed on diets containing either fresh sunflower oil supplemented to a level of 60 (FL) or 240 (FH) mg α-tocopheryl acetate/kg diet, or oxidized oil (approximately 200 meq O_2/kg oil) with no added α-tocopheryl acetate (OS0), or basal levels (OSL) or high levels (OSH). A control group (C) was fed on a reference diet containing tallow supplemented with 10 mg α-tocopheryl acetate/kg diet. After 6 weeks the birds were slaughtered and tissues were removed and stored at -20° until analysis. Thiobarbituric acid-reacting substances (TBARS) were measured by the method of Kornbrust & Mavis (1980).

		TBARS (nmol malonaldehyde/mg protein)							
	Dietary α-tocopherol	Leg muscle		Breast muscle		Liver		Heart	
Group	(mg/kg)	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
C	11	0.50[a]	0.04	0.74[a]	0.03	0.50[a]	0.01	0.80[a,c]	0.08
FL	60	0.70[b]	0.06	1.03[a,d]	0.20	0.61[b]	0.02	0.70[a]	0.04
FH	240	0.63[a,b]	0.06	0.42[b]	0.01	0.59[b]	0.02	0.62[a]	0.05
OS0	2	2.27[c]	0.26	1.77[c]	0.06	2.30[c]	0.44	4.40[b]	0.20
OSL	32	1.14[d]	0.14	1.12[a,d]	0.11	0.59[b]	0.02	0.99[c]	0.07
OSH	211	0.73[a,b]	0.13	0.42[b]	0.02	0.58[b]	0.02	0.64[a]	0.04

[a-d] Mean values in the same column with unlike superscripts are significantly different (*t*-test): $P<0.05$.

TBARS were similar in fresh oil-fed groups at high and low dietary α-tocopherol levels, except for breast muscle. In oxidized oil-fed groups TBARS decreased with increasing dietary α-tocopherol concentrations. At dietary α-tocopherol levels >200 mg/kg, TBARS were similar in fresh and oxidized oil-fed groups, indicating that the effect of oxidized oil was ameliorated.

The results show that relatively low levels of α-tocopherol are sufficient to ensure oxidative stability in fresh oil-fed groups. Although increasing dietary α-tocopherol from 2 to 32 mg/kg diet improved the oxidative stability of oxidized oil-fed groups, this level was not the optimum necessary to ensure adequate tissue stability. Caution should therefore be exercised in the use of oxidized oils in poultry feeds. It must be recognized that dietary α-tocopherol levels should be adjusted to compensate adequately for the increased oxidative stress imposed by oxidized oils.

Izaki, Y., Yoshikawa, S. & Uchiyama, M. (1984). Lipids **19**, 324-331.

Kornbrust, D.J. & Mavis, R.D. (1980). Lipids **15**, 315-322.

Manipulation of the polyunsaturated fatty acid content of pig meat in conformity with dietary guidelines. By C.O. LESKANICH[1], R.C. NOBLE[1] and C.A. MORGAN[2], [1]*Department of Biochemical Sciences, SAC(Auchincruive), Ayr KA6 5HW* and [2]*Department of Genetics and Behavioural Sciences, SAC(Edinburgh), West Mains Road, Edinburgh, EH9 3JG*

Recent health recommendations regarding fat intake have highlighted the importance of consuming polyunsaturated fatty acids (PUFA) in a manner which takes into account the competing physiological roles of the *n-6* and *n-3* PUFA families (British Nutrition Foundation, 1992); furthermore, particular attention is being paid to the C_{20} and C_{22} PUFA. Several studies have demonstrated that an increased *n-3* PUFA content in pig meat can be achieved by a corresponding increase in *n-3* PUFA content of the animal diet (*cf.* Morgan *et al.* 1992). The aim of the present investigation was to obtain information on the optimum length of time required to induce changes in pig meat fatty acid composition which comply with current health advice.

Thirty Large White x Landrace pigs (mean liveweight 29.4(SE 0.6) kg) were randomly assigned to three groups of ten pigs comprising equal numbers of entire males and females. A diet containing 50g soyabean oil plus 10g 'Boost' fish oil (Seven Seas Ltd., Hull)/kg (SFOD) was fed *ad lib.* for 2 (2SFOD), 4 (4SFOD) and 6 (6SFOD) weeks before slaughter after feeding a basal diet containing 50g soyabean oil/kg. 'Boost' oil contained 18.8g eicosapentaenoic acid (20:5*n-3*) and 11.3g docosahexaenoic acid (22:6*n-3*)/100g fatty acids. The major component of soyabean oil was linoleic acid (18:2*n-6*) present at 54.1g/100g fatty acids. Pigs were slaughtered at a mean liveweight of 78.1(SE 2.2) kg. Major lipid fractions and fatty acid compositions of *Semitendinosus*, *Longissimus dorsi*, liver and outer and inner backfat were measured by thin-layer and gas-liquid chromatographic techniques. Tissue cholesterol was estimated colorimetrically.

In the liver, free fatty acid level decreased ($P \leq 0.003$) whilst phospholipid (PL) increased ($P \leq 0.008$) with increasing duration of SFOD. Tissue cholesterol was not significantly affected by treatment. Significant treatment differences in *n-3* PUFA composition occurred in triacylglycerol (TG) and PL fractions of the tissues. The mean content of 20:5*n-3* and 22:6*n-3* (g/100g fatty acids) and total *n-6:n-3* fatty acid ratio of the intramuscular PL are shown in the Table.

	Semitendinosus PL			*Longissimus dorsi* PL		
	20:5*n-3*	22:6*n-3*	Total *n-6:n-3*	20:5*n-3*	22:6*n-3*	Total *n-6:n-3*
2SFOD	1.62	1.43	7.69	1.52	1.32	7.91
4SFOD	1.78	1.75	6.87	1.74	1.65	7.02
6SFOD	2.22	1.87	6.34	2.12	1.96	5.93
SED	0.10***	0.09**	0.32**	0.12**	0.14**	0.41**

Significant difference (by ANOVA): $^{**}P \leq 0.01$, $^{***}P \leq 0.001$

Levels of 20:5*n-3* and 22:6*n-3* were also significantly affected by treatment in TG fractions of *Semitendinosus* (both $P \leq 0.002$), *L. dorsi* ($P \leq 0.001$ and $P \leq 0.005$ respectively) and inner backfat ($P \leq 0.001$ and $P \leq 0.03$ respectively). The total *n-6:n-3* ratio was significantly affected by duration of SFOD feeding and attained the optimum 6:1 ratio in 6SFOD treatment. Results show that by inclusion of dietary fish oil in pig diets the perceived health value of pig meat can be significantly improved in a short space of time both with respect to ratios of *n-6* to *n-3* acids and C_{20} and C_{22} components in particular.

This experiment was funded by the Meat and Livestock Commission.

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Effect of copper and/or vitamin E deficiency on DNA damage in heart and aorta cells of hamsters fed on fish oil or maize oil. By E. TURLEY, V.J. MC KELVEY-MARTIN and J.J. STRAIN, Human Nutrition Research Group, University of Ulster, Coleraine, BT52 1SA

Epidemiological evidence suggesting that fatty fish consumption is protective against coronary heart disease has resulted in the investigation of fish oils as therapeutic agents. While many beneficial effects have been highlighted, some have cautioned that increased consumption of highly polyunsaturated n-3 fatty acids leads to increased oxidative stress in vivo and an increased requirement for dietary antioxidants. If oxidative stress occurs then all cellular components, including DNA, will be susceptible to damage. In the current study the influence of dietary Cu and vitamin E deficiencies on DNA strand breakage, as assessed by the comet assay (Mc Kelvey-Martin et al. 1993), was investigated in heart and aorta cells of hamsters fed on either a maize oil- or fish oil-based diet (150g/kg diet). Fish oil was kindly provided by Seven Seas.

A total of forty-eight male Syrian hamsters were fed ad lib. for 14 weeks on diets as indicated in the Table. Hearts were excised, and small (mg) samples taken, treated with collagenase (EC no.3.4.24.3), washed and mounted in agarose for lysing, electrophoresis, staining and subsequent viewing using a Nikon Optiphot epifluorescence microscope.

The results for each dietary group are shown in the Table.

	Tail moments				Tail length (μm)			
	Heart		Aorta		Heart		Aorta	
Diet Group	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Maize oil (n 7)	18	2	18	3	145	14	149	12
Maize oil, Cu deficient (n 8)	24	2	16	3	173	4	153	7
Fish oil (n 7)	21	4	25	5	161	19	171	16
Fish oil, Cu deficient (n 8)	24	3	13	3	164	15	136	16
Fish oil, vitamin E deficient (n 7)	23	4	18	4	167	10	156	15
Fish oil, Cu and vitamin E deficient (n 6)	22	2	20	3	163	7	171	12

Two way ANOVA indicated no significant difference between the dietary groups in the two indices of cellular damage i.e. tail length and tail moments (a function of the tail length and optical density). These data do not indicate increased DNA damage and, therefore, do not support the concept that increased fish oil consumption results in increased oxidative stress in vivo. However, there was no significant difference in DNA damage under the well-documented oxidative stresses of either dietary vitamin E or Cu deficiency.

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Protective effects of β-carotene and astaxanthin against oxidative stress in vitro. By S.M. LAWLOR and N.M. O'BRIEN, Department of Nutrition, University College Cork, Republic of Ireland

Evidence exists for the role of oxidant damage in the pathology of chronic diseases including cancer and heart disease. Recent studies indicate that certain carotenoid pigments may inhibit free radical damage. β-carotene is an efficient quencher of singlet oxygen and may function as an antioxidant (Burton & Ingold, 1984). Astaxanthin and canthaxanthin, found primarily in crustaceans, act as potent antioxidants in rat liver microsomes (Palozza & Krinsky, 1992). In the present study the ability of β-carotene and astaxanthin to protect against oxidative stress in a cell culture model was assessed.

Primary cultures of chicken embryo fibroblasts (CEF) were cultured in an air-CO_2 (95:5, v/v) atmosphere at 37° in HAM's F10 medium. The cells were oxidatively stressed by exposure to paraquat (PQ; 0.25 mM). Activities of the antioxidant enzymes superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6) and glutathione peroxidase (GSH-Px; EC 1.11.1.9) were measured as indices of oxidative stress.

	SOD (U/mg protein)		CAT (U/mg protein)		GSH-Px (U/mg protein)	
β-carotene (μM)	Mean	SE	Mean	SE	Mean	SE
Control[1]	8.95*	0.43	4.14*	0.08	7.07*	0.97
0	16.31	0.33	10.02	0.42	2.59	0.44
0.5	15.10*	0.20	7.71*	0.21	9.08*	0.69
1.0	9.18*	0.09	6.26*	0.15	7.44*	0.55
10	10.51*	0.20	6.42*	0.09	12.75*	1.80
100	19.59	1.27	10.27	0.04	5.34*	0.34

[1] Control cells containing no paraquat or β-carotene.
* Significantly different from paraquat-treated cells with no β-carotene (unpaired *t*-test): $P<0.05$.

CEF incubated with 0.25 mM-PQ for 18h exhibited increased SOD activity compared with control ($P<0.05$). CAT activity increased ($P<0.05$) and GSH-Px decreased significantly in the presence of 0.25 mM-PQ. No cytotoxicity, as indicated by lactate dehydrogenase (LDH; EC 1.1.1.27) release, was observed at PQ concentrations below 2 mM. Incorporation of added β-carotene (1.0 μM) into 0.25 mM-PQ-treated CEF returned SOD activity to that seen in non-PQ-treated cells. β-carotene (1.0 μM) reduced the CAT activity from that seen in PQ-treated cells and returned the GSH-Px activity to its control value.

At higher concentrations of β-carotene (100 μM) the nutrient appears to be acting as a pro-oxidant in our model system thus reproducing the effects first described by Burton & Ingold (1984). SOD and CAT activities were not significantly different from paraquat-treated cells in the presence of β-carotene (100 μM). Incorporation of astaxanthin (1 nM-100 μM) into the PQ-treated cells protected against PQ-induced oxidative stress at all levels tested. Though known to have good antioxidant properties in *in vitro* systems, little work has been done on the antioxidant potential of astaxanthin in cellular model systems. Our results suggest that at normal partial pressures of O_2 astaxanthin functions as an antioxidant in cellular models.

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Tocopherol content, fatty acid composition and oxidation products in copper-mediated low density lipoprotein oxidation. By B. CORRIDAN and M. J. GIBNEY, Unit of Nutrition and Dietetics, Department of Clinical Medicine, Trinity College Medical School, St. James's Hospital, Dublin 8, Republic of Ireland

Low-density lipoprotein (LDL) highly enriched with linoleate is more susceptible to oxidation than LDL highly enriched in oleate (Reavens, 1991). α-Tocopherol increases the resistance of LDL to oxidation (Esterbauer, 1991). These studies by Esterbauer and Reavens suggest that the fatty acid composition and tocopherol content of LDL may be important factors in determining the susceptibility of LDL to oxidation. The aim of the present study was to investigate the interrelationship between LDL tocopherol content and fatty acid composition and their effects on LDL oxidation in a group of people having more usual ranges of LDL fatty acids and LDL tocopherol.

Fasting blood samples were obtained from nineteen normal, healthy non-smokers. LDL was isolated by ultracentrifugation at 197 000*g* for 24h. LDL (200μg protein/ml) was oxidized at 37^0 for up to 24 h in 5μM $CuSO_4$ in Phosphate Buffered Saline (PBS) (pH 7.4). Portions were withdrawn periodically for analysis of thiobarbituric acid reactive substances (TBARS), tocopherol and fatty acid composition. Conjugated diene compounds (200μg LDL protein/ml in 5μM $CuSO_4$ in PBS at room temperature) were measured spectrophotometrically at 234nm over 24 h.

LDL tocopherol concentration decreased with time; α-tocopherol was consumed more rapidly than γ-tocopherol. The proportion of PUFA in LDL also declined with time, n-6 PUFA declining more gradually than n-3 PUFA. There was an increase with time in the concentration of substances absorbing at 234nm and in TBARS .

The data were examined by multiple regression on three levels. The relationship between tocopherols and PUFA at the initiation of oxidation was examined. The effects of these on some oxidation variables were also investigated. The combined effects of both the initial and changing levels of tocopherol and PUFA on the dynamics of LDL oxidation were examined.

At the initiation of oxidation, tocopherol and PUFA of LDL were not related. Investigation of the effects of only the initial levels of tocopherols and fatty acids on subsequent parameters of oxidation yielded no explanation for (a) the percentage of initial levels of tocopherols remaining at 0.5 and 1.0 h, (b) the rate of formation of conjugated dienes and (c) the concentrations of conjugated dienes and TBARS at 10 and 24 h. Further investigations which take into consideration changes during oxidation show that a combination of initial α-tocopherol levels, initial n-3 PUFA content, rate of conjugated dienes formation and the dynamic PUFA and tocopherol content of LDL are important determinants of TBARS at 10 and 24 h. Similar investigations show that the initial arachidonic acid content of LDL enhances the regression model explaining the levels of conjugated dienes at 10 and 24 h. However, the rate of formation of conjugated dienes, the level of conjugated dienes at the previous time point and the percentage of initial levels of PUFA remaining at time points during the course of oxidation have a stronger influence on levels of conjugated dienes at 10 and 24 h. These results demonstrate that the susceptibility of LDL to oxidation is not fully determined by the levels of PUFA and tocopherol in the ranges found in normal unsupplemented LDL.

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Effect of lutein on oxidation of low-density lipoproteins (LDL) *in vitro*. By M. CHOPRA and D.I. THURNHAM, Human Nutrition Research Group, Department of Biology and Biomedical Sciences, University of Ulster, Coleraine, BT52 1SA

Lutein (3,3'-dihydroxy-α-carotene) is a yellow, xanthophyll carotenoid and is as widely distributed as ß-carotene in green vegetables and fruits (Heinonen *et al* 1989). In Britain blood lutein is 10-40% of total plasma carotenoids (Thurnham & Flora, 1988). Like α-tocopherol, lutein (but not ß-carotene) correlates with cholesterol in blood (Thurnham, 1989) and is an antioxidant in several *in vitro* systems (Chopra & Thurnham, 1993). α-Tocopherol is the main antioxidant in LDL but, in unsupplemented subjects, it does not correlate with LDL susceptibility to oxidation (Dieber-Rotheneder *et al* 1991). Other antioxidants may therefore play a role in protecting LDL *in vivo*.

In the present study we investigated the influence of lutein on copper-initiated oxidation of LDL. Ethanolic lutein (67 μl), following filtration (0.2 μm Nalgene filters), was added to 5ml plasma (20, 40 & 50 nmol lutein/ml) and incubated at 37°for 3h. Plasma was then adjusted to a density of 1.22 g/ml with KBr and LDL prepared by centrifuging for 22 h at 10°and 40,000 rpm (285000 *g*) using a Beckman SW41 rotor and a discontinuous KBr gradient (1.08, 1.05 and 1.00 g/ml) (Dieber-Rotheneder *et al.* 1991). Lutein (Thurnham & Flora, 1988) and cholesterol (CHOD-PAP; Boehringer) concentrations were measured following desalting (Biogel P-6; Bio-Rad), by monitoring formation of lipid dienes following the exposure to 1.67 μmol $CuSO_4$/l (Dieber-Rotheneder *et al.* 1991).

Treatment		Lutein μmol/l Plasma	LDL	Lutein (nmol/mg* total LDL)	Lag phase (min)
None		0.674	0.312	0.078	104
Lutein:	20 nmol/ml	8.645	1.936	0.484	128
	40 nmol/ml	21.134	2.920	0.730	195
	50 nmol/ml	24.172	3.568	0.892	217

*LDL mass calculated as LDL cholesterol multiplied by 3.16 (Dieber-Rotheneder *et al* 1991)

The results showed that increasing the lutein content of LDL delayed the onset of oxidation and extended the lag phase in the copper oxidation test. The increases in LDL lutein achieved were 5-, 8- and 11-fold corresponding to lag phase increases of 20, 88 and 109% and these effects are 10× greater than those of vitamin E (mol/mol). The sigmoidal response suggests a critical concentration of lutein is necessary for maximal effect but this may be an *in vitro* phenomenon since *in vivo* supplementation with vitamin E produces a linear increase in lag phase.

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Relationships between blood antioxidant enzyme activities and measures of oxygen consumption in children and adolescents. By CAROLINE G. EGERTON, M. BARBARA E. LIVINGSTONE, P.G. McKENNA and J.J. STRAIN, Human Nutrition Research Group, University of Ulster, Jordanstown, Newtownabbey, Co. Antrim BT37 0QB

O_2 consumption and antioxidant status were measured in eighty-four healthy subjects (forty-two males and forty-two females) of three age-groups (7-8 years, 13-14 years and 18-19 years) to test the hypothesis that increased O_2 consumption, by producing harmful reactive oxygen species (ROS) at a higher rate, is compensated by an increase in antioxidant enzyme activities.

Superoxide dismutase (*EC* 1.15.1.1, SOD) and glutathione-peroxidase (*EC* 1.11.1.9, GSX-PX) activities were measured automatically on the Cobas-Fara Autoanalyser (Roche), while catalase (*EC* 1.11.1.6, CAT) and caeruloplasmin (*EC* 1.16.3.1, CPL) activities were assayed manually (Aebi, 1984 and Schoinsky *et al.* 1974, respectively). Total energy expenditure (TEE) was estimated by heart rate monitoring and basal metabolic rate (BMR) by indirect calorimetry under standard conditions. Peak O_2 consumption (PVO_2) was determined using the Bruce incremental exercise protocol (Bruce *et al.* 1972). Results were analysed by multivariate analysis. Significant differences between age-groups were tested by least significant difference (LSD) test.

	7 - 8 Year olds				13 - 14 Year olds				18 - 19 Year olds						
	Male		Female		Male		Female		Male		Female		Statistical effects		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Age(A)	Sex(S)	AxS
SOD (U/gHb)	399	27	436	20	436	17	451	11	368	14	424	22	NS	*	NS
Catalase (U/gHb)	34.3	1.9	30.8	2.5	35.6	1.8	31.5	2.8	21.5	2.2	33.9	2.0	*	NS	***
GSH-PX (U/gHb)	26.8	2.1	33.2	1.8	27.1	1.6	30.4	3.1	30.8	2.2	36.1	2.9	NS	*	NS
CPL (U/l)	300	16	289	18	220	15	231	17	173	10	225	17	***	NS	NS
BMR (MJ/d)	4.81	0.1	4.67	0.1	6.21	0.2	5.92	0.2	7.35	0.2	5.58	0.2	***	***	***
BMR (kJ/kg)	195	5.6	195	6.3	144	2.7	119	4.5	108	2.0	96	2.8	***	**	*
TEE (MJ/d)	7.90	0.2	7.14	0.4	12.1	0.8	10.6	0.5	14.2	0.8	9.94	0.4	***	***	**
TEE (kJ/kg)	320	11	296	13	277	15	212	7.0	207	10	173	9.3	***	***	NS
PVO_2 (l/min)	1.34	0.1	1.12	0.1	2.44	0.1	2.36	0.1	4.21	0.1	2.59	0.1	***	***	***
PVO_2/BW (ml/kg/min)	53.8	1.8	46.7	3.0	56.1	1.6	47.1	1.3	61.2	1.2	44.2	1.1	NS	***	*

PVO_2/BW, Peak oxygen uptake/bodyweight; NS, not significant.
Significant statistical effect (MANOVA): $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$.

MANOVA results showed that the age differences in antioxidant enzymes are, in part, consistent with the hypothesis that increased oxidative stress may be compensated by an increase in antioxidant activities. Younger individuals had greater BMR (kJ/kg) and TEE (kJ/kg) values, thus reflecting greater O_2 consumption compared with older individuals, and had significantly greater catalase and CPL activities compared with older age-groups. Sex differences were not consistent with the hypothesis, results suggesting that females are better protected against the toxic O_2 species generated in normal metabolic processes, despite the greater O_2 consumption values seen in males.

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Effects of feeding high-fat diets and subsequent starvation on catalase (EC 1.11.1.6) activity in rat muscle. By J.M. VAUGHAN, S. MURPHY and N.M. O'BRIEN, Department of Nutrition, University College Cork, Republic of Ireland

High-fat diets and conditions such as diabetes and starvation are known to enhance peroxisomal β-oxidation of fatty acids and catalase activity in rat liver. Muscle catalase activity increases during starvation and when hypolipidemic drugs are fed, suggesting a role for muscle peroxisomes in responding to altered lipid metabolism. MacDonald & Swan (1992) demonstrated an increase in both peroxisomal fatty acid oxidation and catalase activity in rat muscle in response to a high saturated fat (lard) diet. Iritani & Ikeda (1982) have reported increased catalase activity in liver of rats fed on a diet high in linoleic acid. It is appropriate, therefore, to determine the effect of feeding diets high in either saturated or unsaturated fat on catalase activity in cardiac and skeletal muscle.

Male Sprague-Dawley rats were trained to meal feed using a fat-free (FF) diet for 9 d. Seven rats were killed to serve as baseline controls and the remaining animals were either fed for 7 d on either a fat-free (FF) diet or high-fat diets (46% energy from fat) containing a highly saturated fat (coconut oil) (SF) or a highly polyunsaturated fat (sunflower seed oil) (UF), or fed on those diets for 5 d and starved for 2 d (SFF, SSF, SUF). The SF and UF diets were offered in amounts calculated to be isoenergetic with the FF diet. Catalase activity was determined by the method of Baudhuin et al. (1963).

	Catalase (μg/g tissue)											
	Gastrocnemius			Soleus			EDL			Heart		
Group	n	Mean	SE	n	Mean	SE	n	Mean	SE	n	Mean	SE
Baseline	7	21	5	7	50	15	4	12	2	7	106	12
FF	4	17[a]	1	4	76[a]	33	4	7[a]	2	2	124[a]	27
SF	4	31[b]	8	3	159[b]	24	4	17[b]	3	7	191[b]	26
UF	4	30[b]	7	4	161[b]	37	4	26[b]	4	5	230[b]	11
SFF	7	55[b]	15	7	122[b]	20	4	31[b]	10	3	208[b]	29
SSF	7	43[b]	9	7	150[ab]	27	4	27[b]	6	4	227[b]	13
SUF	7	46[b]	8	7	171[b]	33	4	38[b]	3	6	275[b]	49

EDL, Extensor digitorum longus

[a,b]Within column means with unlike superscripts differed significantly (ANOVA two-way, followed by LSD test): $P<0.05$.

Compared with rats fed on the fat-free diet (FF), rats fed on either SF or UF diets had elevated catalase activity in all muscles. Similar catalase activities were observed in animals fed on either sunflower seed oil (UF) or coconut oil (SF). Starvation resulted in increased catalase activity in white (EDL) and mixed fibre muscles (gastrocnemius). No significant effects of starvation were observed in red muscle (soleus) or cardiac muscle. Our data indicate that muscle catalase activity is elevated in response to both saturated and unsaturated fat intake.

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Fibre and antioxidant vitamin intakes of women in relation to recommendations for dietary sugars. By C. BOLTON-SMITH , Cardiovascular Epidemiology Unit, University of Dundee, Ninewells Hospital and Medical School, Dundee DD1 9SY

The practical difficulty of achieving a diet low in both total fat and sugars is recognised, since a low fat diet is most easily obtained by substitution of energy by sugars, rather than the more bulky but nutritionally "favoured" starches. The new recommendations (COMA, 1991) distinguish the type of sugars with regard to their potential dental health effects, and set an upper limit forintake of extrinsic sugars of 10 % energy, but a lower limit for the total of intrinsic sugars, lactose and starch (37 % energy).

The fibre and micro-nutrient composition of the diets of low and high sugar consumers according to the COMA definitions have been investigated in 5858 women aged 25-65 who participated in the Scottish Heart Health (Bolton-Smith et al. 1991) and MONICA (Tunstall-Pedoe, 1988) studies. Diet was assessed by food frequency questionnaire (Bolton-Smith et al. 1991), and food sugars were assigned to intrinsic, extrinsic or milk sugar categories based on MAFF guidelines (personal communication): Intrinsic sugar was that present naturally in whole fresh fruits, vegetables and grains, plus 50 % of the analysed sugars present in cooked or processed fruits; Extrinsic sugar (non-milk) was that sugar not naturally occurring within the intra-cellular structure of foods, plus 50 % of the sugar in cooked or processed fruits, and lactose was sugar derived from milk, regardless of its location (i.e. including lactose in chocolate, puddings etc.). The Table shows the mean daily nutrient intakes for women consuming <10 % energy (LES) and 10-30 % energy (HES) from extrinsic sugar and < 37 % energy (LST) and >37 % energy (HST) from starch +lactose+intrinsic sugar.

	Energy (MJ)		Fat (% energy)		Fibre (g)		Vit C (mg)		Vit E (mg)		β-Car (μg)		Rct (μg)	
Diet type	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
LES	7.11‡	1.87	40.5‡	6.1	19.9‡	7.1	54‡	24.7	6.7‡	5.0	3477‡	2283	711	432
HES	8.05	1.99	37.7	5.1	17.6	6.6	47	22.1	6.0	4.5	3202	2275	694	419
LST	7.41*	1.91	40.9‡	5.6	18.6‡	6.2	51‡	23.6	6.6	5.0	3310‡	2219	734‡	439
HST	7.26	2.06	34.1	4.2	24.6	7.8	58	25.9	6.5	4.5	3801	2501	598	397

Vit C, vitamin C; Vit E, vitamin E; β-Car, β-carotene, Rct, retinol. Significant difference between low and high extrinsic sugar or starch groups by analysis of variance on the appropriately transformed data * $p < 0.05$, ‡ $p < 0.001$

Results indicate a higher total energy, and lower fat, fibre, vitamin C, vitamin E and β-carotene intake in those women eating a diet with more than 10 % energy compared with those eating less than 10 % energy from extrinsic sugar. The effect on fat intake may be considered favourable, and no overall effect on vitamin A intake occurs. The lower intakes of vitamin E and β-carotene, while statistically significant, are likely to be of negligible biological significance since, if vitamin E requirements are considered in relation to polyunsaturated fat intake, there is little evidence of deficiency of these nutrients in this population (Bolton-Smith 1990). Lower intakes of fibre and vitamin C may be of more importance with regard to health in this group, however a large proportion of these differences are likely to be due to cigarette smoking. The lower intake of the vitamins and fibre in the low consumers of other sugars and starch are likely to be due to the major sources of intrinsic sugar being fresh fruit and vegetables. Ideally a relatively high sugar diet which assists with the achievement of the low fat dietary goals (Gibney 1990) should be accompanied by a greater intake of fruit and vegetables than is found in Scotland at present.

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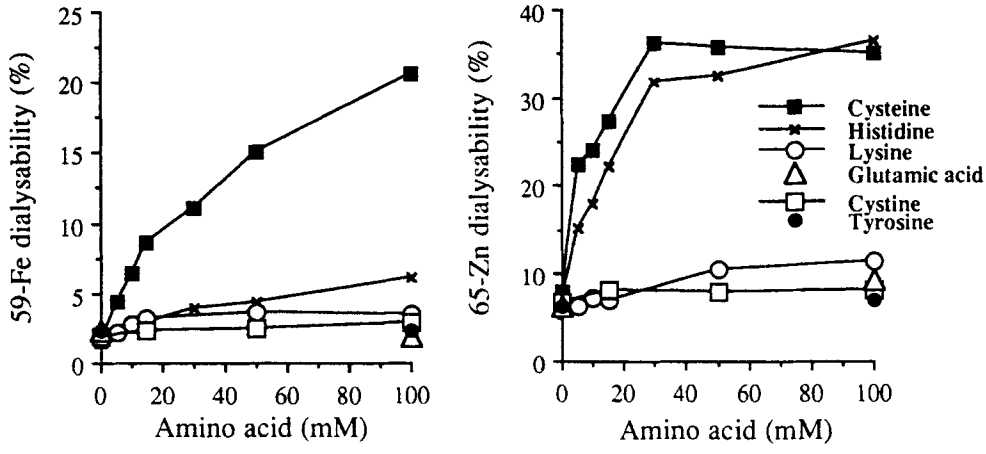
The effects of amino acids on iron and zinc dialysability *in vitro*. By T. BENNETT, C. KEHOE and A. FLYNN, Department of Nutrition, University College, Cork, Republic of Ireland

Certain food proteins enhance the bioavailability of both Zn and non-haem Fe. The exact mechanism by which this enhancement occurs is unclear but it may be due to chelation effects of amino acids in the gastrointestinal tract. The present study compares the effects of a range of amino acids on the *in vitro* dialysability of Fe and Zn.

L-amino acids were added at concentrations of 0-100 mM to a slurry of steam-cooked ground rice in water (50 g/kg). The pH was reduced to 2.0 with HCl and 0.1 mg Fe as $FeSO_4$ and 0.1 mg Zn as $ZnSO_4$, previously labelled with ^{59}Fe and ^{65}Zn respectively, were added. *In vitro* dialysability of Fe and Zn was determined by a modification of the method of Miller *et al.* (1981). The method involves a two-stage simulated gastrointestinal digestion using pepsin (EC 3.4.23.1) and pancreatin, followed by determination of soluble low-molecular-weight (dialysable) ^{59}Fe and ^{65}Zn.



Each data point on the graphs represents the mean of two digestions, each dialysed in duplicate.

^{59}Fe dialysability was greatly enhanced by cysteine, slightly enhanced by histidine and was unaffected by lysine, cystine, tyrosine or glutamic acid. ^{65}Zn dialysability was greatly increased by both cysteine and histidine, whereas lysine, cystine, tyrosine and glutamic acid had little or no effect. Addition of the sulfhydryl blocking agent N-ethylmaleimide, at an equimolar concentration with cysteine, blocked the enhancing effect of cysteine on ^{59} Fe and ^{65}Zn dialysability, indicating that the sulphydryl group of cysteine is responsible for the effect.

These results show that only sulphydryl and imidazole groups are significant enhancers of Zn dialysability and only sulphydryl groups are significant for Fe dialysability, and that amino, carboxyl, and phenolic groups have little effect on the dialysability of Fe and Zn. The increase in Fe and Zn dialysability is probably due to the formation of soluble low-molecular-weight metal-amino acid complexes, although in the case of Fe the sulphydryl group of cysteine may also reduce Fe^{3+} to the more soluble Fe^{2+} form.

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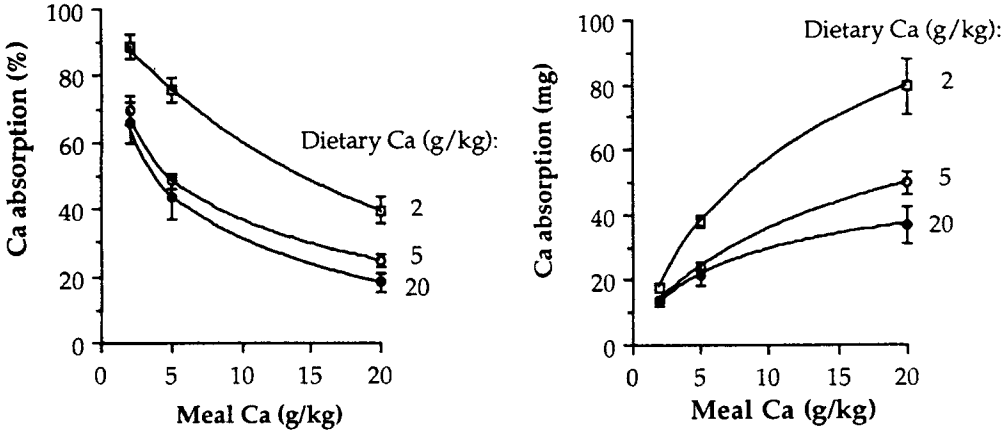
Effect of dietary calcium on calcium absorption from a purified diet in the rat. By K. CASHMAN and A. FLYNN, Department of Nutrition, University College, Cork, Republic of Ireland

The rat is considered to be a useful model for studies on Ca bioavailability since the absorption mechanisms for Ca are similar in rats and humans and many dietary and physiological factors affect Ca absorption similarly in the two species. A detailed understanding of the factors which affect Ca absorption is necessary for the proper design and interpretation of bioavailability studies. Previous dietary Ca intake and meal Ca content are important determinants of Ca absorption. In the present study the influence of previous dietary Ca intake and meal Ca content on Ca absorption from a meal was determined in rats under normal conditions of eating.

Fifty-four 7-week-old male rats, Wistar strain, average weight 180 g, were randomized into three groups of eighteen rats each and fed on a semi-purified diet (AIN-76) containing 2 (low), 5 (normal) or 20 (high) g Ca /kg as $CaCO_3$ for 2 weeks. Each group was then further randomized into three groups of six rats each and fed on a meal (10 g of the same diet) containing either 2, 5 or 20 g Ca /kg as ^{47}Ca-labelled $CaCO_3$. ^{47}Ca was determined in quantitative daily collections of faeces over 7 d and fractional absorption of ^{47}Ca estimated by extrapolating the linear portion (days 3-7) of the plot of log ^{47}Ca retention *v.* time back to the time of isotope administration.



Fractional absorption of meal Ca decreased with increasing previous dietary Ca intake and with increasing meal Ca content. Absolute Ca absorption from the meal decreased with increasing previous dietary Ca, while it increased with increasing Ca content of the meal. The influence of variations in meal Ca content on Ca absorption (load effect) was greater than that of variations in previous dietary Ca intake (adaptive effect), in agreement with findings in humans (Heaney, 1991). Ca as $CaCO_3$ is potentially all available for absorption in rats from a purified diet, subject to Ca need and Ca content of the diet. The findings of the present study emphasize the importance of ensuring that both previous dietary Ca intake and meal Ca content are carefully controlled in studies on Ca bioavailibility.

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Effects of dietary orotic acid on the blood cholesterol content and profile of rats. By J. PEARCE[1,2], and M.J. McASTOCKER[1], [1] Food Science Department, The Queen's University of Belfast, Newforge Lane, Belfast, BT9 5PX and [2] Department of Agriculture for Northern Ireland

It is generally accepted that an elevated blood cholesterol content is a major risk factor in the incidence of coronary heart disease. Yoghurt and fermented milks have been observed to have a hypocholesterolaemic effect in man and experimental animals and it has been suggested (Bernstein et al. 1977; Richardson, 1978; Ward et al. 1982), but not proven, that orotic acid (6-carboxy-2,4-dihydroxypyrimidine - a pyrimidine intermediate involved in nucleic acid metabolism) may be responsible.

Chemically-defined diets containing 0, 2.5, 5.0 and 10.0 g orotic acid/kg were provided *ad libitum* to individually-housed male rats (10 animals per treatment). A significant negative relationship between bodyweight gain and the dietary orotic acid content was noted; feed intake was also reduced by orotic acid. The plasma total cholesterol and high-density lipoprotein (HDL)-cholesterol contents were reduced as the dietary orotic acid content increased (Table) and there was a significant increase in HDL-cholesterol:total cholesterol ratio.

	Dietary orotic acid (g/kg)					
	0	**2.5**	**5.0**	**10.0**	**SEM**	**F ratio**
Plasma cholesterol (mmol/l)	2.30	2.52	1.84	1.50	0.147	P<0.001
HDL-cholesterol (mmol/l)	1.32	1.32	1.27	1.03	0.080	P<0.05

From the above results it is possible that the effects on the blood cholesterol content were due to orotic acid or to differences in food or energy intake. This was examined in a pair-feeding experiment using the same diets with four rats per treatment group. As the dietary orotic acid content was increased from 0 to 10 g/kg diet the plasma cholesterol content was progressively and significantly (P<0.05) reduced from 2.08 to 1.51 mmol/l. In the rats pair-fed to the same dietary intakes as the animals receiving the orotic acid-supplemented diets no reductions were seen. These data show that the effects on plasma cholesterol content are due to orotic acid and not food or energy intake.

Analyses of yoghurt and cows' milk indicated orotic acid contents of approximately 50-65 and 80 mg/l fresh product respectively. To extend the above work orotic acid was added to the diet of rats in amounts which would be equivalent to typical human consumption; this equated to 50 mg/kg chemically-defined diet. In this study individually-housed rats (5 animals per treatment group) were allocated to diets with or without added orotic acid; the duration of the study was 8 weeks. Food intake, bodyweight gain, plasma total cholesterol and HDL-cholesterol were not significantly different between animals receiving diets with or without orotic acid addition.

These results indicate that although orotic acid is hypocholesterolaemic, at the concentration in which it occurs in dairy products it does not have a hypocholesterolaemic effect.

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Effects of dietary yoghurt on cholesterol synthesis and sterol excretion in rats. By J PEARCE[1,2] and M J McASTOCKER[1,1] Food Science Department, The Queen's University of Belfast, Newforge Lane, Belfast BT9 5PX and [2]Department of Agriculture for Northern Ireland

In studies with experimental animals and man it has been reported that milk, especially fermented milk, contains a factor which reduces blood cholesterol content (Mitsuhashi, 1991). We have also observed that dietary yoghurt reduces the plasma total cholesterol content and increases the high-density lipoprotein-cholesterol: total cholesterol ratio in rats (J. Pearce and M.J. McAstocker, unpublished results). However, the above results have not been confirmed in all studies. Other investigations have reported no effects (Massey, 1983; Salmela et al. 1990) or an increase in blood cholesterol (Howard & Marks, 1982; Roberts et al. 1982) with dietary dairy products. Since we observed a hypocholesterolaemic effect, the present work was undertaken to examine the effects of dietary yoghurt on cholesterol synthesis and sterol excretion.

Two groups of five Norway Hooded rats received cereal-based rations with or without freeze-dried low fat yoghurt (80 g/kg diet). These diets were provided *ad lib*. During weeks 16 and 32 faecal samples were collected over a 3 d period and analysed for neutral sterols and bile acids. After 32 weeks the rats received sodium [1-^{14}C] acetate (12.5 μCi) by intraperitoneal injection; 4 h later they were killed and their livers rapidly removed and placed in liquid N_2 prior to the measurement of the radiocarbon incorporated into cholesterol.

The results indicated that dietary yoghurt significantly reduced hepatic cholesterol synthesis from acetate but had no effect on the faecal excretion of neutral sterols and bile acids (Table); for neutral sterols and bile acids only the results after 32 weeks are given.

	Diet		SEM	F ratio
	control	+yoghurt		
[1-^{14}C] acetate incorporation into cholesterol*	8572	3698	726.8	P<0.001
Neutral sterol (mg/g faeces)	2.32	2.47	0.069	NS
Bile acids (mg/g faeces)	9.45	9.14	0.364	NS

* Expressed as disintegrations/min per 4 h per g liver protein.

These results would indicate that the hypocholesterolaemic effect of yoghurt in rats is mediated by a reduction in synthesis with no effects on sterol and bile acid excretion.

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Postprandial triacylglycerol concentration and coagulation factor VII activity. By H.M. ROCHE and M.J. GIBNEY. Unit of Nutrition and Dietetics, Department of Clinical Medicine, Trinity College Medical School, St. James's Hospital, Dublin 8, Republic of Ireland

Elevated coagulation factor VII activiy (FVIIc) is an independent risk factor of coronary heart disease. A strong positive association between factor VII and plasma triacylglycerol (TAG) concentrations suggests that TAG concentration is one of the determinants of factor VII levels. However, it is not clear whether TAG concentration affects factor VII in terms of mass concentration or activation status. Lipolysis of TAG-rich lipoproteins and subsequent transfer of TAG to high-density lipoproteins (HDL) may promote the reactivity of coagulation factor VII.

Postprandial plasma TAG, HDL triacylglycerol concentrations and FVIIc were investigated in twenty four healthy volunteers, following the consumption of a single PUFA fat meal (0.5g fat / kg body weight). Statistically significant postprandial variations were identified using repeated measures ANOVA. Results are presented below as TAG covcentrations in mmol\l and FVIIc expressed as % of reference plasma of known FVIIc.

HOURS	0	2	4	6	8	Least significant difference
Total TAG	0.900	1.024	1.561 [a]	1.288 [a]	0.988	0.142
SD	0.465	0.645	0.942	0.757	0.728	
HDL TAG	0.459	0.322	0.373	0.504	0.543	0.144
SD	0.153	0.155	0.142	0.384	0.546	
Factor VIIc	126.2	128.0	122.2	126.7	129.3	5.796
SD	32.6	30.1	27.2	30.6	32.7	

[a] Significantly different from baseline value (repeated measures ANOVA). $P<0.05$.

Plasma TAG concentrations increased significantly following the test meal and returned to near baseline at 8 h. Fasting plasma TAG concentrations correlated with peak TAG concentrations (r0.935; P=0.02). Fasting plasma TAG concentrations correlated with fasting FVIIc (r 0.481; P=0.001) and with peak postprandial FVIIc (r 0.616; P=0.001). Peak total plasma TAG correlated with peak FVIIc (r 0.507; P=0.01). Following depletion of HDL TAG (2h); FVIIc was positively associated with HDL TAG enrichment. At 4 h FVIIc correlated with HDL TAG concentration (r 0.562; P = 0.005). Factor VII activity 6 h postprandially correlated with HDL TAG concentration at four h (r 0.647; P=0.0008) and at six h (r 0.399; P=0.05).

At 4 h peak plasma total TAG concentrations were reached; then maximal total plasma TAG catabolism occured, HDL was enriched with TAG and coagulation factor VII activity began to increase. There were significant associations of factor VII activity with HDL TAG concentrations of the same time point and previous time point. Thus suggesting that factor VII activity is related to postprandial triacylglycerol metabolism.

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The effects of altered food frequency consumption on plasma lipids in free-living healthy volunteers. By S.A. MCGRATH and M.J. GIBNEY. Unit of Nutrition and Dietetics, Department of Clinical Medicine, Trinity Medical School, St. James's Hospital, Dublin 8, Republic of Ireland

The purpose of this study was to examine how altered food frequency consumption would influence blood cholesterol in free-living subjects following self-selected diets with frequencies of consumption of meals maintained within normal ranges.

From an initial recruitment of eighty-seven men who completed a 7-day food frequency diary, two sub-groups were selected for further study, twelve 'snackers' and eleven 'meal-eaters' representing two extremes of frequency of food consumption. During a stabilization period of four weeks the subjects maintained their usual intake pattern. Nutrient intakes were determined using the diet history method and fasting blood samples were taken for subsequent lipid analysis. At the end of the stabilization period the subjects received individual counselling on how to switch from snacking to meal-eating and *vice versa*, without modifying nutrient intake. During this 4-week switchover period a second diet history and blood sample was taken. Statistical significance of dietary data was investigated with a paired t-test.

The original snackers moved from eating 6.0 (SD 0.8) to 3.3 (SD 0.3) times per day during the stabilisation and switch-over periods respectively. The original meal-eaters increased their number of meals from 3.0 (SD 0.1) to 5.9 (SD 0.9) daily. These meal frequency changes were associated with small but statistically significant changes in nutrient intake (% energy) in the snacking to meal-eating group: protein, 12.8 (SD 1.9) v. 14.0 (SD 1.8) (P= 0.051); fat, 38.6 (SD 3.7) v. 41.0 (SD 4.0) (P= 0.001); saturated fatty acids, 17.5 (SD 2.1) v. 18.5 (SD 7.1) (P= 0.029); alcohol 4.4 (SD 3.2) v. 2.3 (SD 2.4), (P= 0.001), (Wilcoxon signed rank test). No significant changes in diet were observed in the meal-eating to snacking group. The effect of treatment on plasma lipids was determined using cross-over analysis.(Jones and Kenward, 1989)

A significant treatment effect on changing to snacking was seen for total cholesterol (P= 0.038), LDL cholesterol (P= 0.038), HDL/ LDL ratio (P= 0.013) and Apo A-1/ B (P= 0.029). Meal-eateers changing to snacking, who showed no change in dietary intake of nutrients, had a pronounced fall in total cholesterol of 5.00 (SD 0.98) to 4.62 (SD 0.93) mmol/l and in LDL cholesterol 3.37 (SD 1.06) to 2.96 (SD 0.95) mmol/l and a pronounced rise in HDL/ LDL ratio 0.44 (SD 0.2) to 0.51 (SD 0.2) and in Apo A-1/ B ratio 2.17 (SD 0.5) to 2.26 (SD 0.6). Opposite trends were seen for snackers changing to meal-eating hence the significant cross-over effect, but the extent of change was much less and entirely explicable by changes in fatty acid composition as predicted by the Key's equation, (Keys *et al*, 1965).

These results support the concept of regular smaller meals as an effective means of lowering blood cholesterol without changing nutrient intake. The observed cholesterol lowering effect is equivalent to that which can be achieved through oat bran supplementation or through dietary fat manipulation. Therefore the cholesterol reduction attained by alteration of feeding frequency merits attention as a potential therapeutic measure in hypercholesterolaemia.

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This study was funded by Mars (UK)

Is monounsaturated fatty acid rich butter beneficial in the treatment of hyperlipidaemia ? By S.M. RAFFERTY[1*], D. HARRINGTON[2], M.C. CANTON[2], D. O'CALLAGHAN[1], J. MURPHY[2], J.F. CONNOLLY[2], J. HORGAN[1] and M.J. GIBNEY[3], [1]Department of Cardiology, Beaumont Hospital, Beaumont Road, Dublin 9, [2]Teagasc, Moorepark, Co. Cork, and [3]Department of Clinical Medicine, Trinity College, Dublin 2, Republic of Ireland.

The risk of mortality from coronary heart disease is directly related to serum total cholesterol and low-density lipoprotein (LDL)-cholesterol concentrations and is related indirectly to high-density lipoprotein (HDL) levels (Castelli *et al.* 1986; Gordon *et al* 1989). The first line of treatment for hyperlipidaemic patients is dietary modification. This consists of a reduction in total fat intake and saturated fatty acid (SFA) intake to 30% and 10% of total energy intake respectively. The present study examined the effect of dietary modification by manipulation of the fatty acid profile of dairy products. A modification of the bovine diet was made to produce a butter and cheese rich in monounsaturated fatty acids (MUFA) which were not available on the market at that time.

Thirty patients (twenty four males and five females, one excluded) of mean age 56.6 years, all of whom had hyperlipidaemia, had been established on a low-fat diet and had stable lipid levels, participated in the present study. Using a double change-over design, ordinary butter and cheese (A), MUFA-enriched butter and cheese (B) and PUFA-enriched spread and cheese (C) were included in the low-fat diet of the participants for 6 weeks each. Similar amounts of butter and cheese were consumed in each of the three trial diets. Body mass index (BMI), total serum cholesterol, serum triacylglycerols, HDL-cholesterol, LDL-cholesterol, total cholesterol : HDL-cholesterol ratio, HDL_2-cholesterol, HDL_3-cholesterol and lipoprotein (a) were measured before starting the study and at the end of each 6 week intervention period (Intervention A, B and C). A 3 d dietary assessment (using the weighed intake method) was carried out initially and once during each of the three intervention periods.

The administration of the MUFA-enriched butter and cheese resulted in a significant elevation in HDL_3 compared with the preliminary period ($P<0.05$). No other significant changes were observed in serum lipid levels. A number of studies have suggested that both HDL_2- and HDL_3-cholesterol levels are inversely related to the risk of coronary heart disease (Miller, 1987; Stampfer *et al* 1991). The effect of fatty acid modification on nutrient intake was also examined. This illustrated that small changes in fatty acid intake influenced the dietary fatty acid distribution to a statistically significant level. The highest percentage energy from MUFA occurred during intervention B compared with all other periods ($P<0.001$). PUFA (percentage energy) contributed the greatest amount during diet C compared with all other diets ($P<0.001$). Diet A contributed the greatest energy from SFA compared with all other diet periods ($P<0.001$).

The results of the present study show that inclusion of ordinary butter and cheese (A), MUFA-enriched butter and cheese (B) and PUFA-enriched spread and cheese (C) in the low-fat diets of hyperlipidaemic patients had no detrimental effects on lipid profile. The study also showed that inclusion of a modified butter and cheese (MUFA) in the low-fat diet of these patients increased the level of HDL_3-cholesterol. However, further investigation is needed to clarify the relative importance of each of the HDL subfractions to the development of coronary heart disease.

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*Present Address: Department of Preventive Medicine, Education & Research Centre, St Vincent's Hospital, Elm Park, Dublin 4, Republic of Ireland.

An investigation of eating patterns in non-ulcer dyspepsia. By P. CUPERUS[1], P.W.N. KEELING[2], A. CHUA[2] and M. J. GIBNEY[1], [1]Unit of Nutrition and Dietetics, Department of Clinical Medicine, and [2]Department of Gastroenterology, Trinity College Medical School, St. James's Hospital, Dublin 8, Republic of Ireland

Non-ulcer dyspepsia (NUD) or functional dyspepsia is a common occurence. NUD can be defined as dyspepsia where clinical assessment and investigation fail to identify any abnormalities to which the symptoms can reasonably be attributed. Dyspepsia is defined as episodic or persistent symptoms that include abdominal pain or discomfort and which are referable to the upper gastrointestinal tract (Heading, 1991). Previous studies in this department have indicated that patients with NUD may have unusual patterns of meal intake (Mullan, 1993). The present study examines the eating patterns of NUD patients to establish whether these patients have a different eating pattern when compared with healthy age- and sex-matched controls. Fifty patients were recruited from the gastroenterology out-patient clinic and the healthcare centre in St. James's Hospital, Dublin, over an 8 month period, from November 1992 to June 1993. All patients were diagnosed as having NUD by endoscopy and other relevant investigations. Patients and controls were asked to record the time and nature of all foods and beverages consumed over a 7 d period. Patients were also asked to record the time at which dyspeptic symptoms occurred. Information was also gathered about age, employment, smoking habits, weight and height. The average number of eating occasions (all foods and beverages) in one day and the average frequency of breakfast, light meal (small meal, mainly lunch or light evening meal), main meal (largest meal of the day, mainly dinner) and snacks (all foods and drinks consumed outside breakfast, light meal and main meal) in 1 week were calculated. An unpaired Student's t-test was used on the Normally-distributed data (frequency of all eating occasions and of snacking) and the Mann Whitney U-test on non-parametric data (frequency of breakfast, light and main meals). Chi-squared analysis was done on employment status and the distribution of the main meals and snacking over the day.

There was a statistically significant difference in the percentage unemployed between patients and controls (46 v 12) on χ^2-analysis ($P=0.002$). There was no significant difference in the mean (SD) eating frequency per day (5.4(1.0)/5.45(1.1)), the total frequency of consumption of breakfast per week (5.9(1.5)/6.4(0.9)), light meals per week (6.1(0.9)/6.0(1.2)) and main meals per week (6.7(0.5)/6.7(0.7)) and snacking pattern per week (16.6(7.1)/18.9(7.7)) between patients and controls. The results remained the same when unemployed subjects were excluded. The distribution of breakfast over the day was statistically different between patients and controls in both groups, total group and employed subjects ($P<0.001$). Patients tended to have their breakfast later in the morning, while the majority of the controls had their breakfast between 7am to 9am. The distribution of lightmeals ($P<0.05$) and main meals ($P<0.01$) was different between patients and controls, but this difference disappears when only employed subjects were examined. These results show no statistical difference in the frequency of eating, but do show a difference in the distribution of meals during the day. Further study is being carried out to assess the frequency and the distribution of food items and duration of fasts during the day.

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Dietary intake and nutrition concerns in Duchenne muscular dystrophy. By G. BYRNE and S. SUGRUE, Department of Biological Science, Dublin Institute of Technology, Kevin Street, Dublin 8, Republic of Ireland

Duchenne muscular dystrophy (DMD) is a fatal X-linked disease which affects boys. It is characterized by muscle degeneration which causes severe debilitating illness, with death usually by 20 years. The primary aim of the present study was to assess the dietary intake in DMD sufferers (13) by means of a 7 day weighed food record and to assess their carers' perception of nutrition-related problems using an interviewer-assisted questionnaire.

The age range (5-21 years) of the participants was extensive considering the course of the disease process. Their weights ranged from 13.8kg to 63.6kg, the former representing the weight of the oldest participant. The most recent weights available from their medical clinic were used, as actual weights were difficult to obtain. These weights were plotted on the DMD ideal weight-for-age centile chart which are based on an assumed 4% per year decline in muscle mass (Griffiths & Edwards' 1988).

Conflicting theories about optimum dietary intake and weight status exist, with lack of information on actual dietary intake. Energy intakes were very low in this group (63 kJ/kg - 354 kJ/kg) with corresponding low nutrient intakes. Mechanical feeding problems such as chewing difficulties and poor feeding skills were widespread (n 10) but of increased severity in the terminal stages. Fibre intakes were generally low (4.17g/d - 12.5g/d) which, in addition to immobility and low fluid intake as well as disease progression, may predispose to constipation, although this was not a reported problem in this group. Patients at the terminal stages of the disease (n 3) are characterized by low body weights and low basal metabolic rates.

From this small sample, three stages of disease progression are apparent : those who are in the early stages of the disease i.e. still ambulatory ; those who are overweight compared with DMD and normal centile charts ; and those who are extremely underweight (terminal stage) and are at risk of malnutrition. Formulation of dietary guidelines and guidelines for weight control should recognize these three categories and any mechanical feeding problems which may place these boys at increased nutritional risk due to poor energy and nutrient intakes. Measurement of BMR by indirect calorimetry and estimation of total energy expenditure should be established at the various stages of disease progression to facilitate the formulation of dietary advice for boys with DMD.

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Basal metabolic rate and lean body mass in man. By G. J. CUSKELLY and K.M.YOUNGER, Department of Biological Sciences, D.I.T., Kevin St, Dublin 8, Republic of Ireland

The concept of 'active tissue mass' and its possible relationship to body heat production was introduced at the beginning of this century. Several workers studying body mass have noted that healthy adult population samples show good correlations between basal metabolic rate (BMR) and lean body mass (LBM; see Cunningham, 1980). The Douglas bag has not previously been used in investigations to evaluate the relationship between BMR and LBM.

BMR was measured in seventeen healthy volunteers (eight male (M), nine female (F), aged 20-30 years), using the Douglas bag expired air collection system (P.K. Morgan, Rainham, Kent). Basal conditions were adhered to; subjects fasted for 12h preceding the investigation, they rested (lying down) for 30min beforehand, and listened to restful music on a personal stereo during both rest and measurement periods. Subjects were allowed 5 min to familiarize themselves with the mouthpiece system. BMR was measured over a 10min period and extrapolated to 24h (mBMR). Anthropometry, using Holtain skinfold calipers at the four standard sites (biceps, triceps, suprailiac and subscapular), was used to estimate LBM. Reproducibility of anthropometric and energy measurements were checked by carrying out four sets of measurements on one volunteer at one week intervals.

	LBM (kg)		mBMR (kJ/d)		cBMR (kJ/d)		LBM*v.* BMR *r*		LBM*v.* BMR *r*
	M	F	M	F	M	F	M	F	M + F
Mean	61.6	47.7	7351	5832	7422	5925	0.75*	0.92**	0.91**
SD	7.22	7.28	992	803	808	937			

Significance of the correlation coefficient r (t test): * $P < 0.05$, ** $P < 0.001$.

As shown in the table, there was no significant difference (paired t test) between the mBMR measured using the Douglas bag and the cBMR calculated from body weight using the equations of Schoefield *et al.* (1985). BMR varied directly with LBM whereas body weight and surface area did not predict BMR as precisely (r 0.72 and r 0.86 respectively). In the present study BMR was less well predicted by LBM in the males than in the females. One of the male subjects had a body mass index of 32, and was therefore classifiable as obese. It is possible that the regression equations of Durnin & Womersley (1974) that were used to predict body fat from skinfolds may not be as accurate in a group containing obese subjects. Disadvantages of using the Douglas bag include discomfort and alteration in the subjects natural breathing pattern. However, the good agreement of these brief measurements of energy expenditure when compared with predicted estimates suggests that the Douglas bag can be an acceptable method of estimating BMR.

In conclusion, the close correlation between BMR and LBM obtained in the present study supports the notion that active tissue mass determines basal metabolic energy requirements.

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Should nutrient variables be transformed before epidemiological analyses? By H. MILLNS[1], M. WOODWARD[1,2] and C. BOLTON-SMITH[2], [1]Department of Applied Statistics, University of Reading, Earley Gate, Whiteknights Road, Reading, RG62AL, [2]Cardiovascular Epidemiology Unit, University of Dundee, Ninewells Hospital and Medical School, Dundee, DD19SY

The distribution of the intakes of many nutrients is often positively skewed. This fact is frequently ignored and untransformed variables are used in analyses where normality of the nutrient intakes has been assumed. In cases where a transformation has been considered, it is often the logarithm or square root transformation that is automatically used.

Power transformations resulting in approximately symmetrical distributions were obtained for each of the nutrients recorded in the Scottish Heart Health Study, a cross-sectional survey of 10 359 men and women aged 40-59 years which assessed nutrient intakes by food-frequency questionnaire (Bolton-Smith *et al.* 1991). The transformations were methodically chosen for each nutrient (for males and females separately) using the letter values of the variable (Velleman & Hoaglin, 1981).

The effect that transforming nutrient variables before analysis can have on the outcome of a two-group comparison of nutrient intakes in this population is illustrated in the Table. For each nutrient a two-sample t test was used to compare the intakes of those men with evidence of coronary heart disease (from past medical history, the Rose chest pain questionnaire or an ECG; n 1069) with those men with no evidence of heart disease (n 3828). Three similar sets of t tests were carried out after first applying either the recommended letter value transformation, a square root transformation or a logarithmic transformation to the nutrient. The Table shows the different P-values obtained.

Nutrient (Amount /d)	Untransformed	Letter value transformation	Square root transformation	Logarithmic transformation
Energy (kJ)	0.167	0.023 *	0.060	0.018 *
Saturated fat (g)	0.559	0.113	0.195	0.044 *
Polyunsaturated fat (g)	0.616	0.420	0.517	0.438
Protein (g)	0.444	0.123	0.227	0.104
Starch (g)	0.068	0.039 *	0.041 *	0.027 *
Sugar (g)	0.347	0.019 *	0.070	0.007 *
Cereal fibre (g)	0.023 *	0.076	0.044 *	0.105
Vegetable fibre (g)	< 0.001 *	< 0.001 *	< 0.001 *	< 0.001 *
Retinol (μg)	0.788	0.252	0.398	0.149
β-carotene (μg)	0.116	0.030 *	0.030 *	0.004 *
Vitamin C (mg)	< 0.001 *	< 0.001 *	< 0.001 *	< 0.001 *
Vitamin E (mg)	0.972	0.603	0.990	0.844

* P-value = 0.05 or less

The use of a transformation can alter the conclusions derived (at say a 5% significance level). Using the untransformed variable instead of applying the letter value transformation may suggest erroneous significant differences, as for example with cereal fibre, or may obscure significant differences, for example with energy, starch, sugar and β-carotene. In some cases the type of transformation doesn't affect the outcome, while for others it does.

It is not possible to say whether failure to transform a nutrient variable will definitely alter the conclusions obtained from statistical tests that assume normality. However, it is obvious from the results of this work that transforming nutrient variables should at least be considered more carefully in the future than it is generally considered at present.

H.M. is a recipient of an MRC studentship.

Bolton-Smith, C., Smith, W.C.S., Woodward, M. & Tunstall-Pedoe, H. (1991). British Journal of Nutrition 65, 321-335.
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Adequacy of vitamin A and vitamin C intakes in Scotland: the use of probability analyses. By H. MILLNS[1], M. WOODWARD[1,2] and C. BOLTON-SMITH[2], [1]Department of Applied Statistics, University of Reading, Earley Gate, Whiteknights Road, Reading, RG62AL, [2]Cardiovascular Epidemiology Unit, University of Dundee, Ninewells Hospital and Medical School, Dundee, DD19SY

The dietary reference values (DRV) published by the Committee on Medical Aspects of Food Policy (COMA, 1991) were used to estimate the adequacy of vitamin A and C intakes in middle-aged Scots. The nutrient intakes of 5123 men and 5236 women, aged 40-59 years, were assessed by food-frequency questionnaire as part of a cross-sectional survey, the Scottish Heart Health Study (Bolton-Smith *et al.* 1991).

COMA assumes that the requirements of both vitamin A and vitamin C are normally distributed and thus they set three DRV for each vitamin:

(i) estimated average requirement (EAR), the notional mean of the requirement distribution
(ii) reference nutrient intake (RNI), EAR+2(SD)
(iii) lower reference nutrient intake (LRNI), EAR–2(SD),

where SD is the notional standard deviation of the distribution of requirements.

For each vitamin these DRV were used in a probability analysis to determine the inadequacy of intakes (for males and females separately). The method used was a modified version of the probability analysis described by Anderson *et al.* (1982). For the *i*th individual, the probability, $P\alpha_i$, that their intake, α_i, of the vitamin was inadequate (i.e. less than their individual requirement) was given by $P\alpha_i = \text{Prob}\left(Z < \frac{\alpha_i - \text{EAR}}{\text{SD}}\right)$, where Z is a standard normal random variable. The estimated percentage of the population whose intake was inadequate is then $\frac{1}{n}\sum_{i=1}^{n} P\alpha_i$, where n is the sample size. This estimated percentage is given for vitamins A and C in the Table. The percentage of the population with an intake less than the RNI and the percentage with an intake less than the EAR are also given, and illustrate the problem of using a fixed cut point to estimate deficiency levels in a population.

The effect of simple dietary manipulation on population deficiency levels was investigated by hypothetically adding one orange and four 100g portions of green vegetables per week to everyone's diet and then repeating the probability analysis.

					Probability Analysis: estimated percentage with an inadequate intake	
		Sample size (n)	Percentage< RNI	Percentage < EAR	Actual diet	Modified diet
Vitamin A	Males	4838	15.0	4.8	5.7	3.3
	Females	5020	8.6	2.7	3.0	1.5
Vitamin C	Males	5077	30.8	5.7	8.4	0.0
	Females	5197	36.4	11.9	13.7	0.0

It can be seen that estimating deficiency levels from the percentage of the population with an intake less than the RNI gives a worse impression of inadequacy than the probability analysis, whereas the percentage with an intake less than the EAR gives a better impression. The basic problem with a fixed cut point approach is that the variability of the requirements between individuals is not taken into account. A probability analysis overcomes this difficulty.

In this population it is clear that relatively small dietary additions could eliminate vitamin C deficiency and nearly halve vitamin A deficiency (defined according to the COMA (1991) recommendations).

H.M. is a recipient of an MRC studentship.

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A quantitative food frequency questionnaire for the assessment of dietary iron intake in Irish adolescents. By B. C. MULLIN and N. P. KENNEDY, Unit of Nutrition and Dietetic Studies, Department of Clinical Medicine, University of Dublin, Trinity Centre, St James's Hospital, Dublin 8, Republic of Ireland

A number of studies have indicated that dietary Fe intake in a substantial minority of female adolescent British schoolchildren is below recommended levels (Benton & Roberts, 1988; Nelson, 1991). The 1990 Irish National Nutrition Survey (INNS) revealed low mean Fe intakes in female children aged between 8 and 12 years. The assessment of dietary Fe intake in the adolescent group is problematic.

In the present study, a quantitative interviewer-assisted food-frequency questionnaire (FFQ) was developed for the assessment of dietary Fe intake. A photographic food atlas, identical to that used in the INNS, was used with the FFQ to improve estimation of portion sizes. The performance of the FFQ was compared with a 7d weighed food record (7DWR) completed by twenty primary schoolchildren aged 11-13 years (10 boys, 10 girls), after the FFQ. The results are shown in the Table.

Fe intake (mg/d) . . .		7DWR			FFQ		7DWR - FFQ	
	n	Mean	SD	r	Mean	SD	Mean	SD
Boys+girls	20	10.4	2.6	0.68	9.8	2.4	0.6	2.0
Boys	10	11.0	1.7	0.12	9.8	2.3	-1.2	2.7
Girls	10	9.8	3.3	0.98	9.8	2.7	0	0.8

r, Spearman's correlation coefficient.

No difference was found between the methods of determining mean Fe intake in girls. However, this finding concealed mild misclassification by FFQ into adjacent tertiles by 4 of the 10 girls. The estimation of Fe intake in boys was more difficult, as they seemed less able to recall their dietary intake accurately. This was borne out by their tendency to underestimate iron intake by FFQ and by an unacceptable degree of misclassification (six of ten subjects misclassified by FFQ).

In conclusion, the present pilot study suggests that a quantitative FFQ method may not be reliably used with young adolescent boys, although it may be acceptable in estimating dietary Fe intake in young female adolescents.

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